IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Appln. No.	:	09/920,394
Applicant	:	ISIS Pharmaceuticals, Inc.
Filed:	:	August 1, 2001
TC/A.U.	:	1635
Examiner	:	James Schultz
Customer No.	:	36441
Docket No.	:	ISPH-0589
Confirm'n No.	:	4398
Title	:	ANTISENSE MODULATION OF ACYL COENZYME A CHOLESTEROL ACYLTRANSFERASE-1 EXPRESSION

JDS
3-16-04

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Declaration under 37 CFR §1.132

Sir:

I, Susan Freier, a citizen of the United States, residing at 2946 Renault Street, San Diego, CA 92122, do declare and state that:

1. I am an employee of ISIS PHARMACEUTICALS, INC., the assignee of the above-identified patent application. I hold the degree of Ph.D. in Biophysical Chemistry from the University of California, Berkeley in 1976, working in the

laboratory of Ignacio Tinoco, Jr. Subsequent to obtaining my PH.D. I was a post-doctoral fellow in the laboratory of Irving Klotz at Northwestern University (1976-79) and in the laboratory of Douglas Turner at the University of Rochester (1979-85). I have been employed by the assignee of this application for over 13 years as a Research Scientist and Research Director in the Department of Antisense Research. For the past several years, I have directed the Department of Antisense lead identification where we have measured antisense activity of over 150,000 antisense oligonucleotides designed for over 2000 different genes.

2. I make this declaration as an expert in the art of antisense technology in response to the Examiner's rejection of the claims of this invention as being made obvious under 35 USC § 103(a), by a combination of prior art including Taylor *et al.* **1999** *Drug Disc. Today*, 4(12):562-567 (Taylor). Specifically, I make this declaration to rebut the unsupported statement by Taylor at page 565, col. 1, lines 3-11, that screening 3-6 oligomers per target is sufficient to fine one that inhibits any gene with 66-95% efficiency.

3. Taylor is a review article that makes what I believe to be unsupported assertions about the ease of identifying sites on any gene which may be used as target sites by antisense oligonucleotides that, upon binding to the target, can inhibit the gene expression. The determination of target sites on a gene that permit one to identify suitable, highly inhibitory antisense oligonucleotides for

that gene is not a process which can be anticipated to be easy or simple, merely upon the identification of the gene sequence of the target gene. The results of any screening for a target sequence that permits development of an antisense oligonucleotide that can inhibit gene expression at a high level is never an "expected" result.

4. Applicants' assignee is a company that specializes in antisense technology and uses the latest in bioinformatics programs to identify active sites on selected genes. As indicated by the Exhibits A through D below, one may investigate in excess of 70 or more target sequences of a gene without having success in identifying a target site permitting inhibition at high levels. This is true for a number of genes. While it is possible occasionally to identify a target that permits high level inhibition using less than 25 screening sequences, it is never possible to predict reliably ***before*** the screen is performed, what genes will require the use of greater than 70 screening oligonucleotides and what genes will require the use of less than 25 screening nucleotides.

5. For example, Exhibits A and B show a screen performed on a random gene, human tyrosine kinase, non-receptor, 1 in which 80 screening oligonucleotides were employed in a bioinformatics program. Exhibit A identifies the screening oligonucleotides by ISIS number. Exhibit B plots target mRNA level (relative to untreated control cells) vs. ISIS number of the oligonucleotide. The first two bars on the left are negative control oligonucleotides that are not complementary to this or any other known target. The

others are antisense to the target gene. Most oligonucleotides were inactive, i.e., they had no effect on levels of target mRNA. A few reduced targets to 50-60% control, and thus inhibited about 40-50%. None inhibited more than 50%.

6. For example, Exhibits C and D show a screen performed on a random gene, rat urate anion exchanger 1, in which 80 screening oligonucleotides were employed in a bioinformatics program. Exhibit C identifies the screening oligonucleotides by ISIS No. Exhibit D plots target mRNA level (relative to untreated control cells) vs. ISIS number of the oligonucleotide. Most oligonucleotides were inactive, i.e., they had no effect on levels of target mRNA. A few reduced targets to 60% control, and thus inhibited by about 40%. None inhibited more than 40%.

7. This evidence demonstrates that one skilled in antisense oligonucleotide screening cannot *a priori* expect ease of target identification simply by knowing antisense methodologies and the gene sequence of the entire target. In my opinion, Taylor's statements simply are unduly optimistic and are neither accurate nor capable of being supported by the facts of oligonucleotide screening.

8. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like are punishable by

fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date: 1-5-04 By: Susan Freier

Susan Freier

Exhibit A
Application No. 09/920,394
Declaration of Susan Freier

Date: 11/21/2002
Plate #: 8498
Experiment #: 1
Target: Human tyrosine kinase, non-receptor, 1
Experimentor: Eigen Peralta
Notebook: 2258
Page: 63
Cell Line: T-24
Conc. 2' Deoxys 0 nM
Conc. Uniform MOE Gapmers 150 nM
Primer-Probe Set: RTS1356

Well	Isis No.	% Control Target	Range	Mean Range	Oligo Chemistry	Species Oligo Hits	Comments
B03	18078	97.32	32.85	16.42	MOE-Gapmer	0.00	
F08	18078	108.89	15.97	7.99	MOE-Gapmer	0.00	
F03	300751	82.53	7.57	3.78	MOE-Gapmer	Human	
C09	300752	112.88	82.12	31.08	MOE-Gapmer	Human	
F06	300753	102.82	56.72	28.38	MOE-Gapmer	Human	
B09	300754	104.82	33.55	16.78	MOE-Gapmer	Human	
E06	300755	88.95	55.64	27.82	MOE-Gapmer	Human	
E03	300756	81.88	12.31	6.16	MOE-Gapmer	Human	
D08	300757	100.38	25.00	12.60	MOE-Gapmer	Human	
A09	300758	94.87	33.01	16.50	MOE-Gapmer	Human	
C06	300759	58.60	7.54	3.77	MOE-Gapmer	Human	
H08	300760	87.76	42.59	21.29	MOE-Gapmer	Human	
D03	300761	80.83	4.01	2.00	MOE-Gapmer	Human	
A06	300763	142.14	53.94	26.97	MOE-Gapmer	Human	
H05	300764	105.92	47.79	23.90	MOE-Gapmer	Human	
G08	300765	112.55	16.42	8.21	MOE-Gapmer	Human	
E08	300766	86.14	20.83	10.32	MOE-Gapmer	Human	
G05	300768	80.07	15.15	7.57	MOE-Gapmer	Human	
C08	300769	58.32	8.34	4.17	MOE-Gapmer	Human	
F02	300771	79.96	14.20	7.10	MOE-Gapmer	Human	
H07	300772	66.92	8.37	4.18	MOE-Gapmer	Human	
E02	300773	91.33	20.38	10.19	MOE-Gapmer	Human	
D02	300775	69.59	32.38	16.19	MOE-Gapmer	Human	
B05	300776	80.88	29.24	14.62	MOE-Gapmer	Human	
A05	300777	114.18	19.79	9.90	MOE-Gapmer	Human	
H01	300780	94.30	3.90	1.95	MOE-Gapmer	Human	
D07	300782	80.04	54.02	27.01	MOE-Gapmer	Human	
C04	300783	99.68	29.86	14.98	MOE-Gapmer	Human	
F01	300784	90.93	18.98	9.49	MOE-Gapmer	Human	
B04	300785	79.07	18.01	9.01	MOE-Gapmer	Human	
A04	300786	88.32	23.62	11.81	MOE-Gapmer	Human	
C10	300787	114.28	62.22	31.11	MOE-Gapmer	Human	
E01	300788	65.74	5.81	2.90	MOE-Gapmer	Human	
H03	300789	78.73	48.02	24.01	MOE-Gapmer	Human	
B07	300790	76.38	21.35	10.67	MOE-Gapmer	Human	
A07	300791	90.38	8.90	4.45	MOE-Gapmer	Human	
H10	300792	100.49	20.20	10.10	MOE-Gapmer	Human	
H06	300793	94.33	11.65	5.82	MOE-Gapmer	Human	
G09	300794	76.80	27.85	13.93	MOE-Gapmer	Human	
F09	300795	103.60	13.68	6.84	MOE-Gapmer	Human	
G06	300796	89.89	29.28	14.64	MOE-Gapmer	Human	
E09	300797	88.32	30.93	15.46	MOE-Gapmer	Human	
G10	300798	102.51	8.69	4.45	MOE-Gapmer	Human	
D09	300799	79.11	29.89	14.95	MOE-Gapmer	Human	
F05	300800	88.56	28.52	14.28	MOE-Gapmer	Human	
F10	300801	90.48	28.99	13.50	MOE-Gapmer	Human	
C03	300802	103.58	37.97	18.99	MOE-Gapmer	Human	
B08	300803	128.05	48.64	22.82	MOE-Gapmer	Human	
E05	300806	91.28	49.55	24.78	MOE-Gapmer	Human	
A03	300807	72.44	27.47	13.74	MOE-Gapmer	Human	
H02	300808	96.52	50.18	25.08	MOE-Gapmer	Human	
G02	300809	61.92	4.57	2.28	MOE-Gapmer	Human	
C02	300810	62.38	11.64	5.82	MOE-Gapmer	Human	
B02	300811	80.82	3.28	1.64	MOE-Gapmer	Human	Oligo in this well failed QC
G07	300812	87.03	2.29	1.14	MOE-Gapmer	Human	
A02	300813	78.48	25.78	12.89	MOE-Gapmer	Human	
F04	300814	78.24	14.70	7.35	MOE-Gapmer	Human	Oligo in this well failed QC
D10	300815	98.39	80.21	40.11	MOE-Gapmer	Human	
F07	300816	82.86	10.50	5.25	MOE-Gapmer	Human	
E04	300817	87.91	17.43	8.72	MOE-Gapmer	Human	
D04	300818	89.86	13.88	6.94	MOE-Gapmer	Human	
E07	300819	77.60	25.88	12.94	MOE-Gapmer	Human	
D01	300820	95.70	19.05	9.52	MOE-Gapmer	Human	
B10	300821	73.47	8.60	4.30	MOE-Gapmer	Human	
C01	300822	88.88	25.41	12.71	MOE-Gapmer	Human	
G03	300823	109.74	31.02	15.51	MOE-Gapmer	Human	
B01	300824	127.98	11.66	5.83	MOE-Gapmer	Human	
A01	300825	135.91	44.97	22.46	MOE-Gapmer	Human	
A10	300826	106.43	30.94	15.47	MOE-Gapmer	Human	
C07	300827	158.05	22.71	11.35	MOE-Gapmer	Human	
H09	300828	72.00	25.76	12.88	MOE-Gapmer	Human	
B06	300762	65.74	4.61	2.30	MOE-Gapmer	Human/Mouse	
D08	300767	65.04	6.57	3.28	MOE-Gapmer	Human/Mouse	
D06	300770	138.05	76.53	38.26	MOE-Gapmer	Human/Mouse	
C05	300774	55.31	17.75	8.87	MOE-Gapmer	Human/Mouse	
H04	300778	97.18	24.82	12.41	MOE-Gapmer	Human/Mouse	
G04	300779	107.81	38.64	19.32	MOE-Gapmer	Human/Mouse	
G01	300781	118.93	71.89	35.95	MOE-Gapmer	Human/Mouse	
A08	300804	98.90	20.31	10.16	MOE-Gapmer	Human/Mouse	Oligo in this well failed QC
E10	300805	82.14	18.74	9.37	MOE-Gapmer	Human/Mouse	

Exhibit B
Application No. 09/920,394
Declaration of Susan Freier

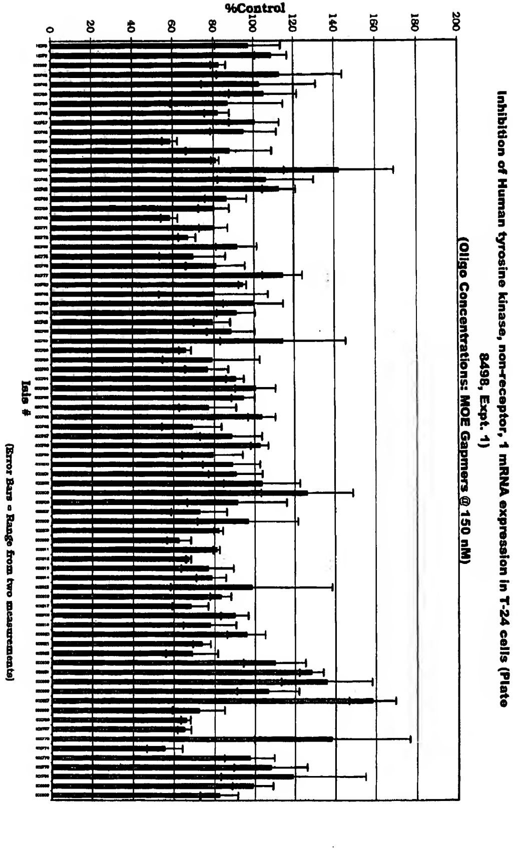

Exhibit C
Application No. 09/920,394
Declaration of Susan Freier

Date: 08/13/2000
Plate #: 9096
Experiment #: 8
Target: Rat urate anion exchang
Experimenter: Joe Barold
Notebook: 2528
Page: 21
Cell Line: Rh-M
Conc. 2' Deoxys 0 nM
Conc. Uniform MOE Gapmers 200 nM
Primer-Probe Set: RT91642

Comments:

Well	Isis No.	% Control Target	Range	Mean Range	Oligo Chemistry	Species Oligo Hits	Hits Amplicon	Comments
B03	18076	68.86	5.34	2.67	MOE-Gapmer	0.00	0	
F06	18076	126.07	20.41	10.20	MOE-Gapmer	0.00	0	
G04	314516	111.93	8.11	4.05	MOE-Gapmer	Mouse/Rat	0	
A02	314517	95.55	39.22	19.61	MOE-Gapmer	Mouse/Rat	0	
A04	314527	61.31	34.96	17.48	MOE-Gapmer	Mouse/Rat	1	
D09	314528	84.16	8.10	4.05	MOE-Gapmer	Mouse/Rat	1	
B06	314533	81.53	3.67	1.83	MOE-Gapmer	Mouse/Rat	1	
G03	314534	93.48	9.76	4.88	MOE-Gapmer	Mouse/Rat	1	
F03	314535	108.62	21.67	10.84	MOE-Gapmer	Mouse/Rat	1	
D01	314536	97.73	7.34	3.67	MOE-Gapmer	Mouse/Rat	1	
F05	314543	95.39	8.83	4.42	MOE-Gapmer	Mouse/Rat	1	
A03	314544	121.37	76.99	38.50	MOE-Gapmer	Mouse/Rat	1	
H02	314545	117.99	39.66	19.94	MOE-Gapmer	Mouse/Rat	1	
C01	314546	91.52	4.42	2.21	MOE-Gapmer	Mouse/Rat	1	
E02	314559	105.45	8.65	4.33	MOE-Gapmer	Mouse/Rat	0	
D08	314560	112.88	5.29	2.64	MOE-Gapmer	Mouse/Rat	0	
H07	314569	99.97	14.28	7.14	MOE-Gapmer	Mouse/Rat	0	
F10	314570	100.57	0.18	0.09	MOE-Gapmer	Mouse/Rat	0	
A05	314571	83.46	31.84	15.92	MOE-Gapmer	Mouse/Rat	0	
G07	314572	102.64	30.97	16.48	MOE-Gapmer	Mouse/Rat	0	
F07	314573	144.06	38.96	19.46	MOE-Gapmer	Mouse/Rat	0	
B02	314574	183.51	14.86	7.44	MOE-Gapmer	Mouse/Rat	0	
H04	314575	130.18	19.43	9.72	MOE-Gapmer	Mouse/Rat	0	
E10	314576	114.71	38.81	18.41	MOE-Gapmer	Mouse/Rat	0	
E07	314577	90.65	3.03	1.61	MOE-Gapmer	Mouse/Rat	0	
D10	314578	100.05	19.57	9.76	MOE-Gapmer	Mouse/Rat	0	
D07	314579	89.54	0.06	0.03	MOE-Gapmer	Mouse/Rat	0	
C07	314580	95.89	7.88	3.93	MOE-Gapmer	Mouse/Rat	0	
G01	314581	110.42	5.79	2.90	MOE-Gapmer	Mouse/Rat	0	
B07	314582	107.90	29.02	14.51	MOE-Gapmer	Mouse/Rat	0	
E04	314583	151.84	46.74	23.37	MOE-Gapmer	Mouse/Rat	0	
A07	314584	68.26	23.84	11.92	MOE-Gapmer	Mouse/Rat	0	
F04	314518	114.08	4.16	2.09	MOE-Gapmer	Rat	0	
H01	314519	102.04	24.10	12.05	MOE-Gapmer	Rat	0	
B10	314520	102.24	18.70	9.35	MOE-Gapmer	Rat	0	
C04	314521	104.79	8.29	4.15	MOE-Gapmer	Rat	0	
H06	314522	69.32	22.84	11.42	MOE-Gapmer	Rat	0	Oligo in this well failed QC
B04	314523	66.09	4.00	2.00	MOE-Gapmer	Rat	0	
G06	314524	70.38	9.03	4.52	MOE-Gapmer	Rat	1	
F09	314525	67.24	13.08	6.54	MOE-Gapmer	Rat	1	
E09	314526	67.83	27.99	13.99	MOE-Gapmer	Rat	1	
F08	314529	82.92	15.04	7.52	MOE-Gapmer	Rat	1	
C06	314530	92.72	6.76	3.39	MOE-Gapmer	Rat	1	Oligo in this well failed QC
C09	314531	76.21	21.18	10.59	MOE-Gapmer	Rat	1	
E01	314532	81.64	6.65	3.33	MOE-Gapmer	Rat	1	
E03	314537	70.14	4.60	2.30	MOE-Gapmer	Rat	1	
D03	314538	93.05	7.88	3.94	MOE-Gapmer	Rat	1	
A06	314539	67.79	14.15	7.07	MOE-Gapmer	Rat	1	
C03	314540	94.03	5.32	2.66	MOE-Gapmer	Rat	1	
H05	314541	100.60	6.83	3.42	MOE-Gapmer	Rat	1	
G05	314542	100.89	10.11	5.06	MOE-Gapmer	Rat	1	
B09	314547	101.88	8.96	4.48	MOE-Gapmer	Rat	1	
G02	314548	92.48	15.09	7.55	MOE-Gapmer	Rat	1	
E05	314549	96.93	6.15	3.08	MOE-Gapmer	Rat	1	
B01	314550	115.15	22.54	11.27	MOE-Gapmer	Rat	1	
A01	314551	125.46	57.81	28.91	MOE-Gapmer	Rat	1	
A09	314552	122.24	45.20	22.60	MOE-Gapmer	Rat	0	
F02	314553	141.49	56.30	28.15	MOE-Gapmer	Rat	0	
D05	314554	154.10	58.76	29.39	MOE-Gapmer	Rat	0	
H08	314555	140.47	4.89	2.44	MOE-Gapmer	Rat	0	Oligo in this well failed QC
C05	314556	92.21	13.01	6.50	MOE-Gapmer	Rat	0	
G08	314557	104.24	24.71	12.35	MOE-Gapmer	Rat	0	
E08	314558	74.21	7.24	3.62	MOE-Gapmer	Rat	0	
B05	314561	76.73	7.15	3.58	MOE-Gapmer	Rat	0	
D02	314562	68.00	8.11	4.06	MOE-Gapmer	Rat	0	
H10	314563	78.30	17.33	8.66	MOE-Gapmer	Rat	0	
C08	314564	89.88	10.37	5.18	MOE-Gapmer	Rat	0	
B08	314565	102.52	0.48	0.24	MOE-Gapmer	Rat	0	
G10	314566	109.74	10.74	5.37	MOE-Gapmer	Rat	0	
A08	314567	84.48	26.56	13.28	MOE-Gapmer	Rat	0	
C02	314568	130.82	31.30	15.65	MOE-Gapmer	Rat	0	
C10	314585	116.81	25.90	12.95	MOE-Gapmer	Rat	0	
D04	314586	117.61	6.15	3.08	MOE-Gapmer	Rat	0	
A10	314587	58.65	0.69	0.35	MOE-Gapmer	Rat	0	
H09	314588	90.33	0.84	0.42	MOE-Gapmer	Rat	0	
G09	314589	94.47	12.54	6.27	MOE-Gapmer	Rat	0	
E06	314590	84.43	0.22	0.11	MOE-Gapmer	Rat	0	
F01	314591	83.56	23.79	11.90	MOE-Gapmer	Rat	0	
D06	314592	66.43	4.11	2.06	MOE-Gapmer	Rat	0	
H03	314593	134.94	24.91	12.46	MOE-Gapmer	Rat	0	

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Exhibit D
Application No. 09/920,394
Declaration of Susan Freier